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PRINCIPAL INVESTIGATOR: Wadie Bahou

CONTRACTING ORGANIZATION: Stony Brook University
Stony Brook, NY 11794-0002

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Wadie Bahou

E-Mail: wbahou@notes.cc.sunysb.edu

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14. ABSTRACT

Human blood platelets play critical roles in normal hemostatic processes and pathologic conditions such as thrombosis (i.e. cardiovascular disease and stroke), vascular remodeling, inflammation, and wound repair. Despite the biological importance of platelets and their intact protein synthetic capabilities, remarkably little is known about platelet mRNAs. The pathogenesis of essential thrombocytosis (ET), a disease of platelet number and function, is poorly understood at the molecular level. The main goal of this project is to build on our preliminary data that suggests that patients with ET have distinct platelet transcript profiles that differ from those of normal platelets. The three main hypotheses to be tested are: (1) patients with ET have mRNA profiles that are distinct from those of normal controls; (2) these differences can be used to elucidate the molecular basis of ET; and (3) these differences can be used to differentiate ET from other causes of thrombocytosis (ET diagnostics). Completion of the specific aims as outlined below should (i) provide considerable insight into the molecular basis of ET, (ii) assist with molecular diagnostics, and (iii) help to devise rational approaches for pharmacological intervention.

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I. INTRODUCTION. Human blood platelets play critical roles in normal hemostatic processes and pathologic conditions such as thrombosis (i.e. cardiovascular disease and stroke), vascular remodeling, inflammation, and wound repair. Despite the biological importance of platelets and their intact protein synthetic capabilities, remarkably little is known about platelet mRNAs. The pathogenesis of essential thrombocytosis (ET), a disease of platelet number and function, is poorly understood at the molecular level. The main goal of this project is to build on our preliminary data that suggests that patients with ET have distinct platelet transcript profiles that differ from those of normal platelets. The three main hypotheses to be tested are: (1) patients with ET have mRNA profiles that are distinct from those of normal controls; (2) these differences can be used to elucidate the molecular basis of ET; and (3) these differences can be used to differentiate ET from other causes of thrombocytosis (ET diagnostics). Completion of the specific aims as outlined below should (*i*) provide considerable insight into the molecular basis of ET, (*ii*) assist with molecular diagnostics, and (*iii*) help to devise rational approaches for pharmacological intervention. These three major hypotheses are the basis for the following specific aims:

Specific Aim I. Validate differences in microarray profiles between normal and ET patients

Specific Aim II. Establish the positive and negative predictive values of these profiles

Specific Aim III. Develop a pilot ET diagnostic microarray chip and evaluate a transcriptome-based approach to the diagnostics of ET.

II. BODY.

A. SPECIFIC AIMS I AND II.

1. APPROACH: Microarray analysis was used to study the molecular basis of essential thrombocythemia using highly-purified platelets isolated from peripheral blood (20 mL) or by apheresis. Differences were validated by quantitative PCR (Q-PCR) and/or protein analyses as necessary.

2. RESULTS: Initial one-way ANOVA identified 170 genes that were differentially expressed, the majority of which (141) were up-regulated in ET platelets; only 29 genes were down-regulated in ET compared to normal platelets. Analysis of the smaller subset of platelet-restricted genes demonstrated that only 13 genes were differentially-expressed (12 up-regulated, 1 down-regulated) in ET. A disproportionate number of upregulated genes encoded proteases or protease inhibitors (*HPSE*, *MMP1*, *SERPINI1*), a class of proteins known to be associated with tumor invasiveness and metastases. The single down-regulated gene (*HSD17B3*) was present in all normal samples and absent in all ET platelets. *HSD17B3* belongs to an extended family of 17BHSDs retaining oxido-reductase activity toward discrete substrates, and encodes an enzyme (type 3 17β-hydroxysteroid dehydrogenase) previously described as testis-specific. This enzyme is known to catalyze the penultimate step in testosterone biosynthesis. Using a functional assay of testosterone generation, we demonstrated that platelets retained 17βHSD3 activity, with nearly 10% of the capacity found in mouse testis, providing evidence for the first non-testicular source of this enzyme. Transcripts for two additional members of this family were found in human platelets, one of which (*HSD17B12*) was upregulated in the initial cohort of patients studied. Subsequent Q-PCR results were entirely concordant for all individuals studied, demonstrating that *17BHSD12:17BHSD3* transcript ratios reliably distinguished ET from normal patient platelets in all samples studied to date (N=20; 6 apheresis samples, 14 peripheral blood samples; $p < 0.0001$). Furthermore, these differential patterns of *HSD17B* expression appeared unrelated to the development of thrombocytosis *per se*, but rather, were restricted to the ET phenotype.

a. Changes to Specific Aims 1 and 2: NONE.

b. Planned experIments for subsequent funding period. During the next funding period, we will continue to document differences in androgenic function, comparing ET to normal and reactive

thrombocytosis platelets. For these experiments, we have currently synthesized peptides corresponding to both HSDs, and will use these to probe for differences at the protein level. Additionally, we are currently expressing both HSD cDNAs into heterologous systems to precisely quantify androgen conversion. There are no barriers to the completion of these studies.

B. SPECIFIC AIM III: Development of a diagnostic platelet oligonucleotide microarray chip

1. RESULTS (Chip design and fabrication). A well-characterized 70-mer oligonucleotide gene set was used as the starting point for our gene chip. The Qiagen probe set contains 34,580 optimized 70-mer oligonucleotides, representing 24,650 genes and 37,123 transcripts. The design is based on the Ensemble (www.ensembl.org/) Human 13.31 Database and the Human Genome sequencing project, and directly deals with alternative splicing variants using common, partial common, or transcript oligonucleotides. The oligonucleotide probe set comes with complete annotation including Gene Ontology, oligonucleotide chromosome coordinates, comparative genomic analysis, and other functional annotations (i.e InterPro, OMIM, etc.). For our purposes, the final list was generated by data-mining of the original 14-chip analyses (*refer to* **Fig. 1**), and incorporated the following gene cohorts: (*i*) a group of platelet-restricted genes with no expression in leukocytes (*N=126*); (*ii*) the preliminary group of discriminatory genes distinguishing between thrombohemorrhagic ET phenotypes (*N=71*) [**note that this subset is known to include 50 leukocyte-expressed transcripts,** and may be sufficient for parallel leukocyte profiling studies]; (*iii*) the list of genes with platelet expression > leukocyte expression by 10-fold (*N=285*); and (*iv*) the list of genes with leukocyte expression > platelet expression by 10-fold (*N=43*) [additional leukocyte "contamination" control]. Further, we have now analyzed the relative gene expression of the platelet list and found that the expression patterns logically follow a Poisson distribution. To date we have printed 100 slides and quality-controlled the first ten with excellent reproducibility in spotting (**Fig. 1**). After removal of duplicates, the final list contains 432 genes which clearly co-segregate by cell-type as demonstrated *in silico (see Appendix Gnatenko, et.al.*., Blood (2006)]. The chip may be readily modified as more informative genes are identified.

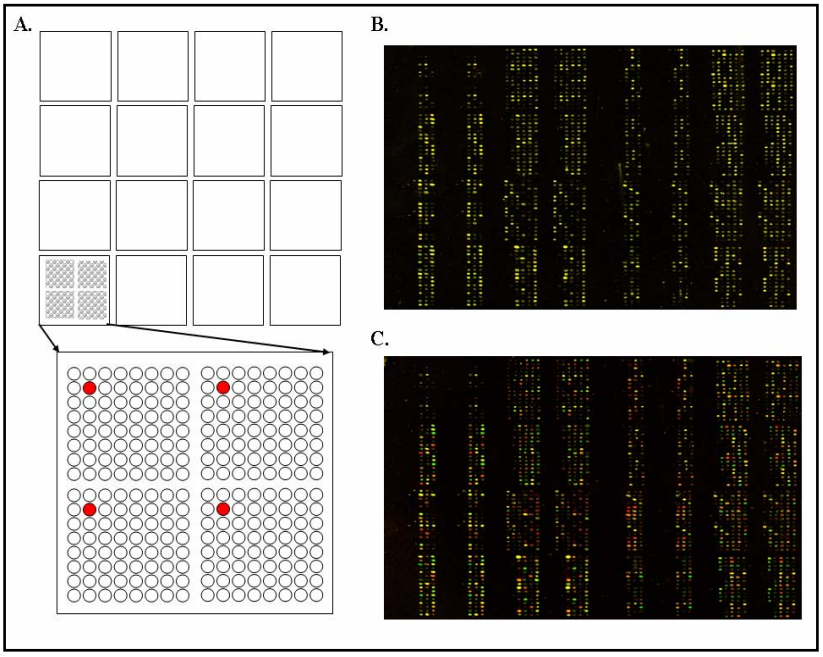
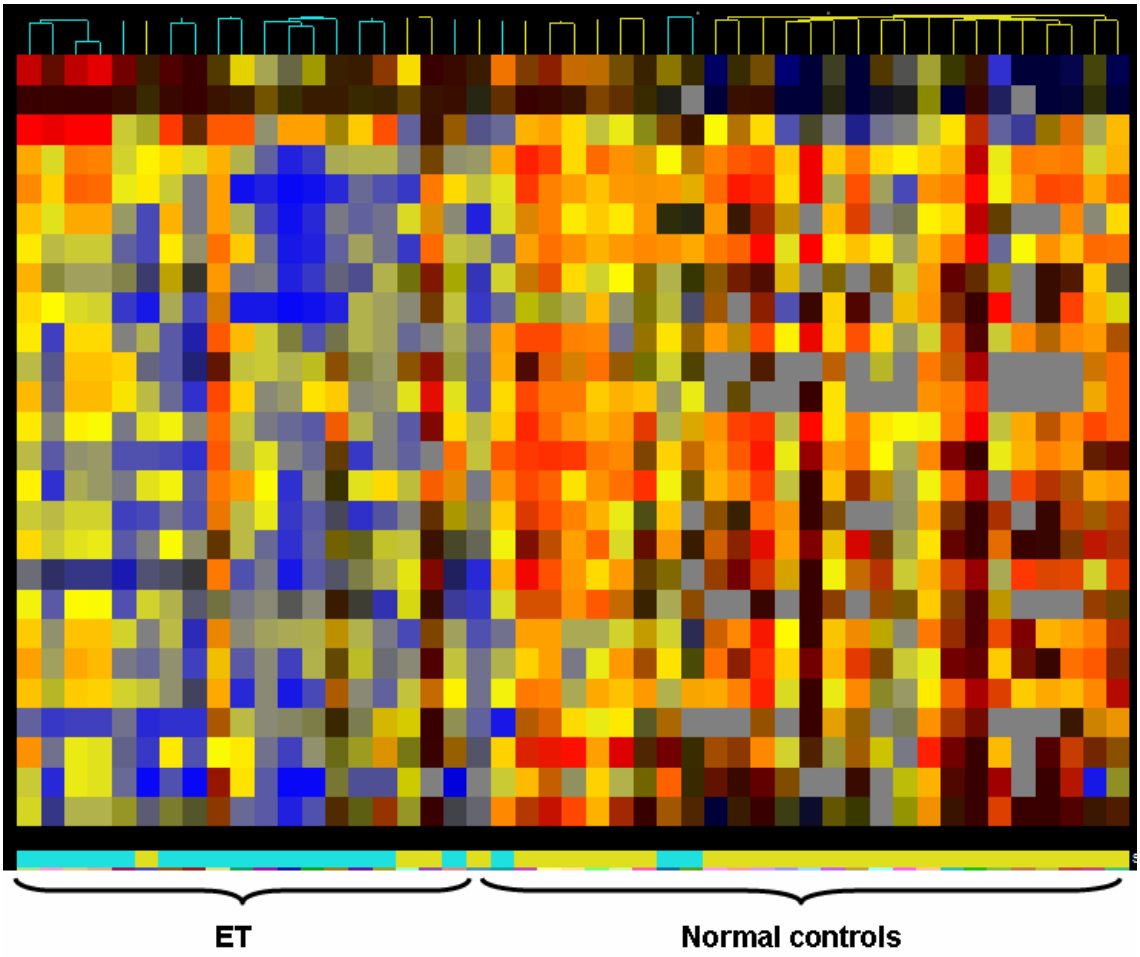


Fig. 1. Oligonucleotides (ODNs) representing the 432-member gene list (along with positive [ribosomal RNAs, housekeeping genes, etc.) and negative [i.e. *Arabidopsis*] controls) were synthesized as 70-mers and used for spotting onto glass slides[1]. ODNs are each represented 4 times/slide (represented as red dots in *Panel A*) as a means of obtaining reliable, integrated expression median values; the final chip contains 2,132 oligonucleotides. For these experiments, two samples are differentially labeled (i.e. either Cy5- or Cy3-), followed by hybridization onto the gene chip. Note that co-hybridization of Cy5- or Cy3-labeled Universal reference RNA (Strategene) provides clear evidence for successful spotting, labeling, and hybridization as demonstrated by the yellow signals from the majority (if not all) of the spotted oligonucleotides (*Panel B*). When we repeated these experiments using platelet (Cy5):human reference RNAs (Cy3), completely different patterns were evident (*Panel C*), all as predicted.

a. Application of the novel platform to ET. Based on these results, we subsequently studied 28 normal patients (13 females, 15 males) and 19 ET patients (13 females, 6 males) using our uniquely-fabricated oligonucleotide chip; in all situations, RNA was isolated from gel-filtered peripheral blood platelets (15 – 30 mL starting blood volume), amplified, hybridized, and processed as outlined above. After correction for the false discovery rate using the Benjamini and Hochberg adjustment, results demonstrated *no gender-related differences between normal controls* for the genes represented on the chip (0/432). In contrast, 26-28 genes

were differentially expressed when comparing ET to normal, using either one-way ANOVA ($p < 0.05$, N=28 genes), or a t-statistic that incorporated intersample variability in expression ratios (ranked by the magnitude of the test statistic numerator)[55,76]; using the latter calculation 26 genes were differentially expressed, with complete overlap with the 28-gene subset identified using ANOVA. As shown in **Fig. 2**, unsupervised hierarchical clustering demonstrates distinct variation in gene expression patterns between the two defined phenotypes (*i.e.* ET [blue] *vs.* normal [yellow]); *note that 41/47 patients are correctly classified by genotypic profile*. Thus, these data clearly establish that **our fabricated chip representing (*i*) a new platform, (*ii*) new patient cohorts, and (*iii*) a new methodology, i.e. Cy3/Cy5 co-hybridization) clearly can be applied for more extensive gene profiling studies in larger patient cohorts.**



b. Changes in Specific Aim 3: None.

c. Planned experiments for subsequent funding period. In the next funding period we will continue our validation studies of the chip, delineating its ability to distinguish ET from other causes of (reactive) thrombocytosis. We will identify a discriminating oligonucleotide profile, and use this to screen additional patients as a means of validation.

III. KEY RESEARCH ACCOMPLISHMENTS.

- First demonstration that platelet microarray studies can be applied to a human platelet disorder
- First demonstration that ET platelets have a profile distinct from normal platelets
- First demonstration that a diagnostic assay may be available to distinguish ET from normal platelets
- Initial fabrication, characterization, and validation of a platelet-diagnostic oligonucleotide chip

IV. REPORTABLE OUTCOMES.

A. Manuscripts accepted and/or published

- Gnatenko, D. and **W. Bahou**. Recent advances in platelet transcriptomics. Trans. Med. Hemotherapy 33:217-226, 2006

• Gnatenko, D., P. Perrotta, and **W. Bahou**. Proteomic approaches to dissect platelet function: half the story. Blood 108:3983-3991, 2006.

• **Bahou, W**. Megaprofiles provide big insights into platelet function. Blood 109:3120-3121, 2007.

• Senzel, L., D. Gnatenko, and **W. Bahou**. The platelet transcriptome and cardiovascular disease. Future Cardiology, *in press*

B. Newly funded awards

R21HL086376 (Bahou) Project period: 9/1/06 - 8/31/08
NIH/NHLBI
Integrated molecular profiling of the thrombohemorrhagic phenotype

C. Pending awards

"*Genetic dissection of the platelet thrombohemorrhagic phenotype*" (NIH/NHLBI)

V. CONCLUSION: We have made considerable progress in all aims of the research, specifically in (*i*) providing the proof-of-principal that ET has unique mRNA transcript profiles, (*ii*) that these profiles can be used to develop potential diagnostic tests for the disease, and (*iii*) in identifying a novel steroidogenic pathway in normal and platelets. Furthermore, we have now created a unique platelet microarray chip that is being developed for more robust profiling. During the subsequent funding cycle, we will continue to validate these initial observations by recruiting a broader cohort of subjects, and initiating preliminary studies to dissect the role of steroidogenic pathways in megakaryocyte proliferation and/or proplatelet formation (thrombopoiesis).

VI. REFERENCES. SEE SECTION IV. Reportable Outcomes (*above*)

VII. APPENDICES.

• Gnatenko, D. and **W. Bahou**. Recent advances in platelet transcriptomics. Trans. Med. Hemotherapy 33:217-226, 2006

• Gnatenko, D., P. Perrotta, and **W. Bahou**. Proteomic approaches to dissect platelet function: half the story. Blood 108:3983-3991, 2006.

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VIII. SUPPORTING DATA. As outlined in Figures 1 and 2 (*above*) and in the appended articles, the platelet oligonucleotide chip provides the requisite tool to screen large populations to optimally establish normal profiles and delineate any inter-racial variability (if it exists). Methods to differentiation ET from RT are currently in place and will proceed using both PCR-based technology for select genes, and the oligonucleotide chip.

Proteomic approaches to dissect platelet function: half the story

Dmitri V. Gnatenko, Peter L. Perrotta, and Wadie F. Bahou

Platelets play critical roles in diverse hemostatic and pathologic disorders and are broadly implicated in various biological processes that include inflammation, wound healing, and thrombosis. Recent progress in high-throughput mRNA and protein profiling techniques has advanced our understanding of the biological functions of platelets. Platelet proteomics has been adopted to decode the complex processes that underlie platelet function by identifying novel platelet-expressed proteins, dissecting mechanisms of signal or metabolic pathways, and analyzing functional changes of the platelet proteome in normal and pathologic states. The integration of transcriptomics and proteomics, coupled with progress in bioinformatics, provides novel tools for dissecting platelet biology. In this review, we focus on current advances in platelet proteomic studies, with emphasis on the importance of parallel transcriptomic studies to optimally dissect platelet function. Applications of these global profiling approaches to investigate platelet genetic diseases and platelet-related disorders are also addressed. (Blood. 2006;108:3983-3991)

Introduction

Human blood platelets play important roles in fundamental biological processes, including thrombosis, inflammation, wound repair, and stroke. Although they are anucleate and lack nuclear DNA, platelets retain small amounts of megakaryocyte-derived mRNA.[1,2] Platelets also contain rough endoplasmic reticulum and polyribosomes, thus retaining the capacity for protein biosynthesis from cytoplasmic mRNA.[3] Quiescent platelets display minimal translational activity, although platelet activation leads to the rapid translation of preexisting mRNA,[4] with the release or derivation of platelet-secreted proteins, cytokines, exosomes, and microparticles.

The traditional paradigm that platelet mRNA content is invariant and gradually declines with cell senescence was challenged when signal-dependent pre-mRNA splicing was identified in platelets.[5] Signal-dependent splicing provides a mechanism for altering the repertoire of translatable messages in response to cellular activation/stimulation. Furthermore, platelets have essential components of a functional spliceosome and selected unspliced pre-mRNAs. These spliceosomes retain a unique ability to splice pre-mRNA in the cytoplasm (as opposed to the typical nuclear location), a capability not described in any other mammalian cell. This discovery emphasizes that the molecular mechanisms of platelet function cannot be optimally dissected without accurate platelet transcript profiling.

Modern postgenomic, high-throughput approaches allow integrated studies of molecular components (at the RNA and the protein levels) involved in cell function. Platelets represent an attractive, simplified model for these studies because they lack nuclear DNA and because their genome consists of a small subset of megakaryocyte-derived mRNA transcripts. This complete pool of platelet RNAs is significantly smaller than the transcriptome of a nucleated cell.[6] The entire pool of platelet proteins constitutes the platelet proteome: the initially static, but functionally dynamic, protein interactions that occur with platelet activation. In this review, we focus on recent applications of proteomic and transcriptomic technologies to dissect platelet function in normal processes and pathologic disorders.

Platelet transcript profiling: transcriptomics

Modern approaches to transcript profiling

The development of global transcript profiling technologies, such as microarray and serial analysis of gene expression (SAGE),[7,8] provided novel methodologies for dissecting platelet function. Microarray analysis adapts artificially constructed grids of known DNA samples such that each element of the grid probes for a specific RNA sequence; these are then used to capture and quantify RNA transcripts.[9] Microarray platforms developed to date represent closed transcript profiling systems—that is, they detect only those transcripts that correspond to specific probes imprinted on the chip. Transcripts without corresponding probes are not detected. Recent technological advancements allow accurate whole genome transcript profiling and are capable of detecting alternatively spliced transcripts.[9]

SAGE represents an open transcript profiling system that can detect any transcript within the SAGE library. Classical SAGE[10] relies on the observation that short (less than 10 bp) sequences (tags) within 3′-mRNAs can stringently discriminate among the genes that constitute the human genome. Differentially expressed genes can be identified in a quantitative manner because the frequency of tag detection reflects the steady state mRNA level of the cellular transcriptome.[10,11] Genes expressed at low levels (less than 0.01% of total mRNA) can be identified by SAGE. Modified SAGE protocols have been devised to provide more definitive identification by using longer tags, identify low-abundant transcripts efficiently through subtractive SAGE techniques, and amplify small amounts of mRNA starting material.[12-14]

From the Department of Medicine and the Program in Genetics, State University of New York, Stony Brook, NY; and the Department of Pathology, West Virginia University, Morgantown, WV.

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Analyzing the platelet transcriptome

Initial characterization of platelet-derived mRNA transcripts was achieved by constructing platelet-specific cDNA libraries[15] and through single-gene polymerase chain reaction (PCR)[2] technology. To date, a limited number of microarray experiments using platelet-derived mRNAs have been published; these studies generally agree on platelet transcript quantitation and gene expression patterns.[6,16,17] Furthermore, it is clear that this approach provides an efficient means to identify novel genes and proteins functionally expressed in human platelets.[6] Not surprisingly, platelets retain fewer transcripts—ranging from approximately 1600 to 3000 mRNAs—than those found in nucleated cells[6,17,18] (Table 1). This limited number of platelet-expressed transcripts presumably represents the lack of ongoing transcription in the anucleate platelet.

Given that most microarray protocols are semiquantitative, microarray findings must be validated by other techniques such as quantitative polymerase chain reaction (Q-PCR). The combination of 2 complementary transcript profiling techniques, microarray and SAGE, demonstrated that 50% to 89% of platelet RNA tags are mitochondrial transcripts presumably related to persistent mitochondrial transcription in the absence of nuclear-derived transcripts.[19] The overrepresentation of mitochondrial transcripts in platelets is considerably greater than that of its closest cell type (skeletal muscle), of which 20% to 25% are mitochondrial SAGE tags.[20] Thus, although SAGE clearly has advantages in cellular transcript profiling, its applicability in platelet diseases appears limited. Nonetheless, the relative enrichment of mitochondria-derived transcripts does not interfere with platelet microarray studies.

The feasibility of analyzing platelet transcripts from a single platelet donor with as little as 50 ng total platelet RNA, or approximately 40 mL whole blood, has been demonstrated.[16] Reliable mRNA amplification was validated by Q-PCR and by parallel hybridization of amplified and nonamplified RNA samples. In this study, gene profiling results were reproducible for 9815 of 9850 represented genes, providing initial proof that this approach can be applied in platelet-related human diagnostic studies starting from small sample volumes. Recently, microarray analysis was used to identify genes that are differentially expressed in several platelet-related diseases (see "Global profiling to study platelet-associated disorders," below). These studies clearly establish the feasibility of platelet transcript profiling in identifying differentially expressed genes, characterizing novel platelet-expressed genes, and elucidating the molecular signature of a disease with potential application for platelet diagnostics.

MicroRNA profiling of platelets

MicroRNAs (miRNAs) are a highly conserved class of short, noncoding RNAs that regulate gene expression during cell differentiation, proliferation, and apoptosis. To dissect regulatory pathways that control megakaryocytic differentiation, miRNA expression profiling was performed on in vitro–differentiated megakaryocytes derived from $CD34^+$ hematopoietic progenitors.[21] Several miRNAs were identified, with the subsequent demonstration that miR-130a targets *MAFB*, a transcription factor that is up-regulated during megakaryocytic differentiation and induces the glycoprotein IIb (GPIIB) gene *ITGA2B*. Moreover, the up-regulation of miR-101, miR-126, miR-99a, miR-135, and miR-20 was documented in megakaryoblastic leukemic cell lines compared with in vitro–differentiated megakaryocytes and $CD34^+$ progenitors. These data suggest an important regulatory role of miRNAs during megakaryocytopoiesis; however, a role for miRNAs in thrombopoiesis remains unestablished.

Limitations and perspectives of transcript profiling

Major limitations of modern transcript profiling approaches include reliable and reproducible detection of low-abundant transcripts, feasibility of truly quantitative transcript profiling, bulky and complex data processing, and (in the case of platelets) limited amounts of RNA. Furthermore, accurate platelet transcript profiling requires stringent attention to purification methodologies because a single nucleated cell (ie, leukocyte) contains considerably more mRNA than a platelet.[22] It has become evident that the platelet transcriptome is complex and dynamically controlled at different levels, including regulation by miRNAs,[21] signal-dependent pre-mRNA splicing,[5] and translational control pathways such as mammalian target of rapamycin (mTOR).[4] Despite significant progress in microarray chip design, accurate transcript profiling still requires validation by Q-PCR or other techniques. Efficient mRNA amplification,[16] development of more sensitive whole genome microarrays (which detect alternatively spliced transcripts),[9] and enhancements to bioinformatics software should obviate some of these restrictions.

Platelet protein analyses: proteomics

Modern proteomic techniques

The proteome is the full set of proteins expressed by a genome under a particular set of environmental conditions.[23] The strategy for platelet proteomic analysis generally incorporates platelet fractionation, protein separation, and tryptic digestion methodologies followed by protein identification (Figure 1). Improvements in each of these steps enhance the sensitivity and accuracy of protein identification. Proteomic experiments may begin with protein separation (either in-gel or non-gel); alternatively, the entire mixture of protein can be digested before protein identification (for reviews, see Perrotta and Bahou,[24] de Hoog and Mann,[25] Aebersold and Mann,[26] and Steen and Mann[27]). The availability of the human genome sequence coupled with advances in bioinformatics, computer technology, and mass spectrometry (MS) provide for large-scale, robust, and automated analyses of the cellular proteome.

Table 1. Platelet microarray studies

Reference	No. genes studied	No. present/no. marginal	No. present	No. arrays	Platelet source
Gnatenko et al[6]	12 599	1500/2147	NR	3	Apheresis
Sauer et al[101]	22 200	~1668/3562*	NR	11*	Apheresis
Bugert et al[18]	9 850	NR	~1526	6	Concentrates
McRedmond et al[17]	12 599	NR	2928	1 (in duplicate) from 23 pooled donors	Blood (50 mL)

Numbers of genes studied were the numbers of probes (genes) represented on individual microarray slides.
NR indicates not reported.
*Includes normal and essential thrombocythemic platelets.

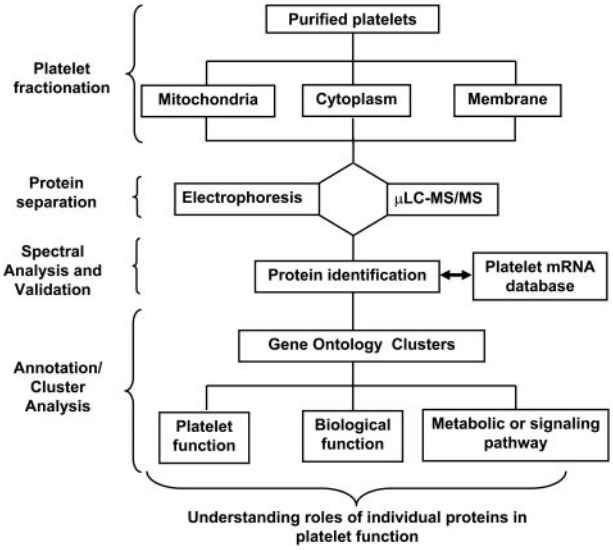

Figure 1. Schema outlining a general approach to platelet proteomic analysis. An ideal experiment generally incorporates platelet subfractionation methods with sophisticated mass spectrometric techniques and computational analyses to elucidate platelet biological functions.

Several mass spectrometry technologies have been developed, including electrospray ionization (ESI-MS),[28] matrix-assisted laser desorption/ionization (MALDI)[29,30] with variations such as ESI-TOF (time of flight) and MALDI-TOF, liquid chromatography coupled to tandem mass spectrometry (μLC-MS/MS), and multidimensional protein identification technology (MudPIT).[31,32] Self-assembled monolayers (SAMs) using trypsin attached to gold have been used to identify 107 platelet proteins, thus establishing the feasibility of proteomic biosensors.[33] More recently, several techniques have been devised to directly compare cellular proteomes, including isotope-coded affinity tags (ICATs)[34] and isobaric tags (iTRAQ)[35-37] coupled to μLC-MS/MS.

Quantitative proteomic[38] techniques are used not only to estimate protein abundance but also to compare cell proteomes. These strategies involve labeling peptides derived from individual samples with isobaric tags (ie, same mass, distinct tags) that fragment into different reporter ions on collision-induced dissociation (CID). Peak area ratios of the reporter ions are used to quantify relative peptide abundance.[39] iTRAQ technology is potentially superior to ICAT-labeling strategies, which detect only cysteine-containing peptides and typically discard peptides that have been posttranslationally modified.[34,39] Other quantitative techniques, such as carboxyl termini labeling of platelet-derived tryptic peptides with oxygen-18, have been used to study the differential expression of platelet proteins.[40]

Limitations of proteomic techniques: sensitivity and the dynamic range

The platelet proteome is subject to rapid changes in response to external signals. Accordingly, accurate and comprehensive platelet protein profiling requires strict attention to technical details to ensure reproducibility and to minimize protein losses resulting from proteolysis, sample preparation, and protein isolation.

Modern proteomic approaches are limited by large differences in the concentrations of the most and least abundant cellular proteins (approximately 5-log difference). Because data acquisition using tandem MS (MS/MS) is triggered by ion abundance levels,[41] peptides derived from more abundant proteins may obscure those derived from less abundant proteins. Thus, this technique can be biased against low-abundance ion signals. In yeast, the dynamic range is insufficient to effectively sample low-abundance proteins of less than 100 copies/cell.[42] Other technical limitations in proteomic techniques include detection of proteins with extremes in pI and molecular weight[43] and membrane-associated or -bound proteins.[44]

Accurate platelet protein profiling requires that platelets be stringently purified of contaminating erythrocytes, leukocytes, and plasma proteins. Table 2 summarizes typical platelet RNA and protein yields obtained from 10 mL peripheral blood, adapting a 2-step purification procedure that incorporates centrifugation, gel filtration, and immunodepletion.[6] Although the amounts of total platelet RNA (approximately 1 μg) and protein are sufficient for most experiments, detection of low-abundance proteins cannot be enhanced because methods for protein amplification (unlike gene-based PCR technologies) are nonexistent.

This issue of protein sensitivity is further highlighted in Table 3, which delineates platelet RNA and protein abundance distributions through composite microarray studies[6] or results from a representative platelet proteomic experiment with μLC-MS/MS spectral counts as estimates of relative protein abundance.[41] Although this distribution of platelet transcript abundances follows a near-normal distribution, the relative protein abundances are distributed differently. Most detected proteins are identified with 1, 2, or 3 peptide hits per protein. Indeed, these results are in agreement with another study in which 62.6% of proteins were identified by a single peptide, with most of the peptide hits corresponding to highly abundant platelet proteins.[45] These data suggest that platelet protein identification using available proteomic technologies has limited ability to quantify peptides with low spectral counts[17] and that protein identification is skewed toward more abundant cellular proteins.[46,47] Although the number of "one-hit wonders" may potentially be reduced by the better separation of proteins before MS identification, it is unclear whether these limitations can be addressed through other quantitative proteomic approaches.[34-37]

Table 2. Total RNA and protein yields from peripheral blood

	Platelet yields, × 10^9				
	Blood, 10 mL	PRP*	Purification†	Yield	Sensitivity, copies/cell
Total RNA	3.1	2.1	1.0	1 μg	<0.1‡
Protein	—	—	—	2 mg	12§

— indicates not applicable.
*Centrifugation step (platelet-rich plasma isolated by centrifugation).
†Gel filtration with or without $CD45^+$ leukocyte depletion.
‡Calculated for microarray analysis after amplification step.
§Calculated based on silver stain detection limits of 1 ng for a 50-kDa protein.

Table 3. Distribution of transcript and protein abundance

Relative abundance, decile	Transcripts, %	Proteins, %	Average no. of peptide hits per protein
1	20.6	85.1	5.8
2	28.4	7.8	39.2
3	22.0	2.8	66.1
4	9.9	2.1	95.8
5	10.6	1.4	111.0
6	2.8	0	—
7	1.4	0	—
8	2.1	0	—
9	1.4	0	—
10	0.7	0.7	257

In this prototype experiment, solubilized platelet proteins were analyzed by μLC-MS/MS, and protein abundance was quantified from the number of peptide hits per individual protein (in this experiment, peptide hits per protein ranged from 1 to 257). Relative protein abundance was determined by the number of peptide hits per protein, and rank-ordered proteins were assigned to deciles based on abundance (1, least abundant; 10, most abundant). The percentage of proteins per decile and the average number of peptide hits per protein are also delineated. Note that most proteins (>85%) were skewed toward those of low abundance, represented by a small number of peptide hits, though transcript expression is distributed more evenly (abundance of corresponding transcripts was determined as previously reported[90]).

— indicates not applicable.

Preanalytical variability in platelet proteomics

The methods used to isolate and prepare protein samples affect the results of platelet proteomic studies. For example, findings may vary when platelet proteins are precipitated with ethanol or trichloroacetic acid.[48] Delays in sample processing and type of anticoagulant used to collect blood samples may also influence the results of MS identification of platelet proteins.[49] Similarly, studies of the low-molecular–weight (less than 15-kDa) peptidome demonstrate that early and gentle platelet separation is crucial for obtaining reproducible identification of platelet-released peptides and proteins.[50] Other studies have emphasized the importance of protease inhibitors and uniform sample storage in improving data reproducibility.[51] These studies demonstrate a clear requirement for standardization of preanalytical variables to ensure comprehensive, reproducible, and comparative (laboratory-to-laboratory) platelet proteomic analyses.

Platelet proteomic analyses

Platelet proteomic studies can be grouped into 2 distinct yet overlapping subcategories, proteomic analyses of quiescent platelets (the static platelet proteome) or activated platelets (the functional platelet proteome) (Figure 2). Whole proteomic strategies identified many platelet proteins, although subsequent approaches to dissect the changes that occur with platelet activation focused on platelet fractions (membrane proteins) or functional end points (eg, phosphorylation patterns, microparticles) in response to external stimuli. Such subproteomic studies use the same technologies but provide a more detailed analysis of function over time.

Quiescent platelets: the static platelet proteome. Initial studies of the platelet proteome focused on characterizing proteins in resting platelets through combinations of 2-dimensional electrophoresis (2-DE) and in-gel protein detection using monoclonal antibodies.[52-54] Although successful, these approaches were limited by the dynamic range of platelet proteins, coupled with the cost and sensitivity of immunodetection. Similar techniques were subsequently applied to establish a platelet protein map and to characterize tyrosine-phosphorylated proteins in resting platelets.[55] In this study, cytosolic protein fractions were separated by 2-DE, and phosphorylated proteins were immunodetected using antiphosphotyrosine antibodies and MALDI-TOF. One hundred eighty-six protein spectra corresponding to 123 unique proteins were identified through this approach. By making use of immobilized pH gradient (IPG) strips to facilitate the one-dimensional gel separation, 286 platelet proteins were identified in the acidic range (pI range 4-5).[56] A more comprehensive profiling of platelet proteins (pI range 5-11) identified 760 protein features corresponding to 311 different genes, resulting in the annotation of 54% of the 2-DE (pI range 5-11) proteome map.[57]

Combined fractional diagonal chromatography (COFRADIC) is a non-gel–based technique in which peptide sets are sorted in a diagonal reverse-phase chromatography system through a specific modification of their side chains.[58,59] This technique was used to identify 264 platelet proteins present in cytosolic and membrane fractions; the dynamic range spanned 4 to 5 orders of magnitude of protein concentration. Modifications of this technology identified a core set of 641 platelet proteins,[45] the largest platelet protein set reported to date. These proteins were

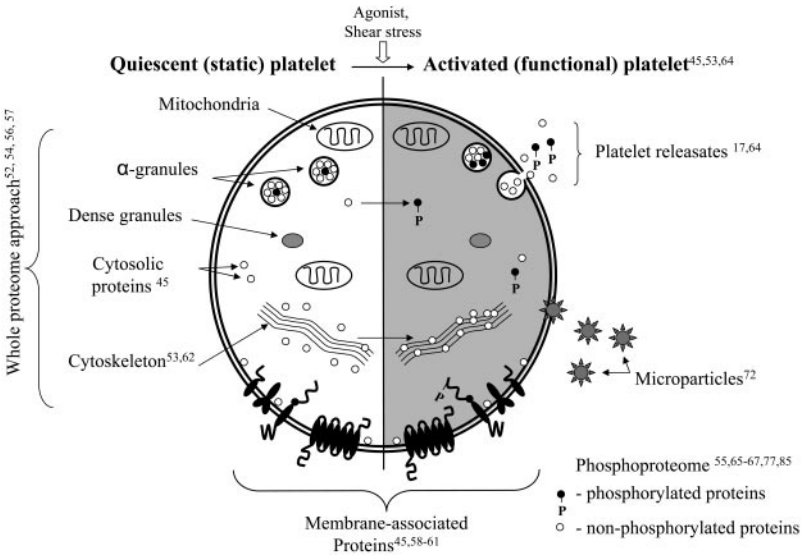

Figure 2. Schema of platelet ultrastructure integrated with proteomic studies. The unshaded panel delineates the proteomic studies involving quiescent platelets. The shaded panel represents those studies focusing on activation-dependent platelet end points (eg, microparticles, exosome/releasates). Superscript numbers refer to references.

classified using the Gene Ontology database, revealing that 16% were membrane proteins (see "Membrane proteins," below), and 64% were present in the cytoskeleton, endoplasmic reticulum, mitochondria, cytosol, or Golgi apparatus. Interestingly, up to 20% of proteins were classified as nucleus restricted. Given that platelets are anucleate, these proteins presumably arise from megakaryocytes during thrombopoiesis; their function in platelets is unclear.

Membrane proteins. The study of platelet integral membrane proteins and surface receptors through traditional 2-DE techniques is limited by the low solubility of these proteins, their association with the platelet membrane, their high molecular weight, and the potentially limited number of diagnostic tryptic cleavage fragments. In addition, the presence of highly abundant cytoskeleton proteins such as actin obscures less abundant platelet membrane proteins.

Two groups have adapted proteomic approaches for the study of platelet membrane proteins.[45,60] Although applications using COFRADIC[45] predicted the presence of 87 putative helix-spanning membrane proteins, a more focused analysis of the platelet membrane proteome was pursued by enriching membrane proteins before protein identification through μLC-MS/MS. In this study, 2 distinct solubilization methods were used to reduce the overrepresentation of cytoskeleton proteins, allowing for the aggregate detection of 233 established or putative transmembrane proteins.[60] Finally, a combination of microarray and mass spectrometric techniques has been used to identify novel membrane proteins that signal during platelet aggregation and to characterize proteins that become phosphorylated on tyrosine or serine residues on platelet aggregation.[61]

Activated platelet proteome: activation-dependent platelet complexes. An early study of activation-dependent responses in platelets identified 27 proteins that were translocated to the actin cytoskeleton during platelet activation.[62] Subsequently, a related study that focused on characterizing static protein complexes as a prelude to dissecting activation-dependent complexes was undertaken in accordance with a novel 2-DE technology that combined 1-dimensional separation of monomeric and multimeric proteins in their native state followed by a 2-dimensional denaturing step.[63] In total, 63 platelet proteins were identified, among them the integral membrane proteins GPIbβ, CD9, and CD36 and the fibrinogen receptor $\alpha_{IIb}\beta_3$. Mitochondrial proteins were detected in the membrane fraction, several of which were organized into known complexes.

Proteins secreted during platelet activation. Proteomic strategies have been used to identify protein subsets secreted during platelet activation. Such strategies resulted in the initial identification of 82 secreted proteins[17]; this was followed by a more robust analysis of the platelet secretome.[64] With the use of thrombin-stimulated platelets, a combination of MALDI-TOF and MudPIT identified more than 300 released proteins.[64] Thirty-seven percent of these proteins were previously known to be released from platelets, whereas another 35% were reported to be released from other secretory cells. The remaining 28% of proteins were not known to be released by any cell type. Moreover, several of the secreted proteins have been previously identified in human atherosclerotic lesions but absent in normal vasculature. In this study, protein profiling resulted in the identification of potential targets for future drug development (ie, secretogranin III, cyclophilin A, and calumenin) and shed light on possible mechanisms of platelet adhesion that contribute to the development of atherosclerosis.

Platelet phosphoproteome. Tyrosine phosphorylation plays a key role in the activation-dependent regulation of protein function, signal transduction, and other complex cellular processes. Several studies have used phosphotyrosine-dependent antibodies and proteomic technologies to dissect intracellular signaling events that occur with thrombin activation, consistently demonstrating an ability to identify discrete proteins, subsets of which had been previously undescribed.[65,66] By using the thrombin receptor activating peptide (TRAP) to specifically activate proteinase-activated receptor 1 (PAR1), 62 differentially phosphorylated proteins were detected, 41 of which were identified by μLC-MS/MS.[67] Interestingly, 8 of these had never been described, and the protein repertoire was shown to originate from 31 genes, highlighting how alternative splicing expands the platelet proteome. Although these initial studies established the feasibility of using proteomic approaches to dissect ligand-dependent phosphorylation changes,[68,69] it is likely that technical advances will further enhance its robustness.[70]

Platelet microparticles. When platelets are activated in vivo, 2 types of membrane vesicles are released, microparticles (which bud from the plasma membrane) and exosomes.[71] Microparticles range in size from 0.1 to 1.0 μm, whereas exosomes are smaller and range from 40 to 100 nm. Platelet microparticles are abundant, exert procoagulant activity, and play hemostatically critical roles in several clinical disorders, including heparin-induced thrombocytopenia and immune thrombocytopenic purpura. A recent proteomic study provided the first panoramic overview of microparticles, identifying 578 proteins that constitute this subcellular proteome.[72] As expected, many of these represented well-characterized platelet proteins. Surprisingly, 380 of 578 proteins had not been previously described in platelet proteomic studies, suggesting these platelet fragments have a unique protein composition.

Integrating platelet transcriptomic and proteomic studies

Recent evidence from mathematical modeling studies demonstrates the need to delineate mRNA and protein expression levels to optimally map intracellular networks. When applied to nucleated cells, these integrated platforms demonstrate some correlation between transcript and protein expression.[73] In platelets, this relationship is even more complex because platelets lack a genome and ongoing RNA transcription, display minimal translational activity, have the capacity for signal-dependent translation of selected mRNAs[4] and inducible (activation-dependent) transcript splicing,[5] absorb select plasma proteins, and contain residual megakaryocyte-derived mRNAs and proteins.

Initial attempts to correlate platelet mRNA and protein profiles have been described.[17,74] These studies demonstrate that up to 69% of secreted and cytosolic proteins were detectable at the mRNA level, suggesting relatively good correlation between proteomic and transcriptomic data in the study of end points of detection and identification.[17] The authors concluded that despite the absence of gene transcription, the platelet proteome is mirrored in the transcriptome, and transcriptional analysis predicts the presence of novel proteins in the platelet. The lack of detailed quantitative correlations limits the ability to compare platelet gene and protein levels with those of nucleated cells.[73]

Global profiling to study platelet-associated disorders

Platelet proteomic studies

Glanzmann thrombasthenia. GT is an autosomal recessive disease characterized by the complete absence or a marked reduction in the $\alpha_{IIb}\beta_3$ heterodimeric complex.[75,76] Compared with normal platelets, GT platelets exhibit a low tyrosine phosphorylation profile, confirming the key role of functional $\alpha_{IIb}\beta_3$ in the initiation of protein tyrosine phosphorylation.[77] Several proteins displayed

attenuated thrombin-dependent phosphorylation kinetics despite normal initial phosphorylation rates. Similar results were obtained by inhibiting thrombin aggregation of control platelets with $\alpha_{IIb}\beta_3$ antagonists or in the absence of stirring. These results suggest that tyrosine phosphorylation of specific proteins is dependent on thrombin activation during early and late steps of $\alpha_{IIb}\beta_3$ engagement in aggregation. No other clear differences were identified between normal and GT platelets.

Ischemic stroke. Genetic risk factors have been identified in patients with thrombophilia, though associations are the strongest for patients with venous thromboembolic disease.[78] Thus, whereas hyperhomocysteinemia and antiphospholipid/anticardiolipin antibodies are stroke risk factors,[79] congenital thrombophilic states (factor V Leiden and prothrombin gene mutations; protein C, protein S, and antithrombin III deficiencies) account for a disproportionately small subset of ischemic stroke,[80] with the greatest risk in younger persons.[81] In contrast, differential expression of platelet proteins may favor platelet activation and thrombus formation. The best evidence in support of this observation stems from correlative studies involving platelet cell surface glycoprotein receptors such as $\alpha_2\beta_1$, in which higher expression levels are associated with increased collagen binding,[82] and greater risk for ischemic heart disease in homozygotes, especially smokers.[83] Similarly, platelet membrane polymorphisms have been linked to stroke in small studies, but the evidence is not strong, and it is most significant in young patients.[84]

A phosphoproteomic comparison of platelets from healthy controls and stroke patients was completed using antiphosphotyrosine antibodies.[85] In unstimulated platelets, a discrete subset of proteins displayed significantly greater tyrosine phosphorylation in 85% of the 20 stroke patients studied than in the healthy controls. Additionally, the authors identified other tyrosine-phosphorylated bands in the stroke platelets that were absent in the resting platelets of the control patients. Although these results may have some prognostic merit in monitoring patients with cerebrovascular insufficiency,[85] they provide no specific protein identification and fail to distinguish cause from effect in dissecting proteomic differences that pathogenetically define platelet-associated stroke risk.

Toxicology. Benzene exposure is an established risk factor for acute myeloid leukemia and may play a role in other human blood diseases.[86] Surface-enhanced laser desorption ionization-TOF MS (SELDI-TOF MS) was used to compare the blood serum proteome of 40 shoe factory workers who experienced well-characterized occupational exposure to benzene with the blood serum proteome of unexposed controls to identify potential biomarkers of benzene exposure.[87] Three low-molecular–weight proteins were consistently down-regulated in exposed subjects. Two of these proteins were identified as platelet-derived CXC chemokines, platelet factor 4 (PF4), and connective tissue–activating peptide III (CTAP III). These findings suggest that lower expression of these proteins may serve as a potential biomarker of early benzene toxicity.

From the transcriptome to the proteome

With today's technology, it is feasible to adapt transcriptomic analysis to predict differences in the proteome, to validate these differences between normal and diseased platelets, and to exploit these differences to develop modern diagnostic tests.[19,74,88]

Direct platelet transcriptomics: essential thrombocythemia as a paradigm. The most compelling evidence that transcript profiling can distinguish diseased from normal platelet profiles is based on studies of essential thrombocythemia (ET) that were conducted with megakaryocytes and platelets.[89,90] ET is a myeloproliferative disorder characterized by increased proliferation of megakaryocytes, elevated numbers of morphologically normal circulating platelets, and considerable thrombohemorrhagic events.[91] Microarray profiling has been used to analyze platelet transcripts of 6 patients with ET and 5 healthy controls.[90] Initial analysis demonstrated different molecular signatures capable of distinguishing normal platelets from ET platelets. Moreover, ET platelets collectively expressed higher numbers of individual transcripts than normal platelets but considerably less distinct transcripts than nucleated cells.[6] Statistical analysis revealed 170 genes that were differentially expressed between ET and normal platelets, most (141 of 170 genes) of which were up-regulated in ET platelets.

Among differentially expressed genes, the transcript encoding 17β-hydroxysteroid dehydrogenase type 3 (*HSD17B3*) was dramatically reduced in ET platelets. This enzyme, not previously characterized in human platelets, belongs to a larger family of *17BHSD*s that play a role in normal and abnormal testosterone biosynthesis.[92] Transcripts for 2 other *17BHSD* family members were identified in platelets; they are *HSD17B11* and *HSD17B12*. Absence of *HSD17B3* transcript expression was evident in all 6 patients with ET. These changes occurred concomitantly with elevated transcript levels of *HSD17B12* in the same patient subgroup. In contrast, the expression of *HSD17B3* in normal platelets was accompanied by negligible *HSD17B12* expression. At the protein level, HSD17β3 enzyme activity was demonstrated in platelet lysates, confirming that human platelets retained the capacity to catalyze the final step in gonadal testosterone synthesis. These data demonstrate the potential of using transcriptomics to lend focus to proteomic studies.

Indirect transcriptomic approaches of platelet disorders. *Gray platelet syndrome.* Gray platelet syndrome (GPS) is a rare platelet disorder manifest by bleeding, thrombocytopenia, and a distinct lack of α-granules.[93,94] Recently, microarray analysis was used to study molecular mechanisms involved in GPS, focusing on fibroblasts because these cells may be involved in the transition to myelofibrosis evident in subsets of patients with the disorder.[95] By comparing microarray profiles of normal and GPS fibroblasts, the up-regulation of various proteins (fibronectin 1, thrombospondins 1 and 2, and collagen VIα) was demonstrated. Overexpression of fibronectin and thrombospondin 1 was confirmed at the RNA level by Northern blot analysis, whereas fibronectin overexpression was confirmed at the protein level by immunostaining. Although this study fails to address the role of megakaryocyte-derived proteins in the development of myelofibrosis, it does suggest that distinct protein subsets may play a role in the progression to myelofibrosis seen in some GPS patients.

Polycythemia rubra vera. Like ET, polycythemia rubra vera (PRV) is a myeloproliferative disorder associated with thrombotic complications.[96] Although the molecular basis for enhanced thrombotic risk remains unknown, defects affecting the platelet thrombohemorrhagic balance are envisioned; however, ancillary leukocyte dysfunction linking the inflammatory response to risk for thrombosis cannot be excluded. To date, microarray studies have been restricted to PRV leukocytes to the exclusion of platelets.[97-99] Furthermore, limited correlative proteomic studies validate the differences seen by microarray or stratify transcriptomic differences by thrombotic phenotype. Although further correlative studies are needed, PRV platelets, like ET platelets, may have a distinct proteomic profile that could help explain the pathogenesis of thrombosis in this hematopoietic disorder.

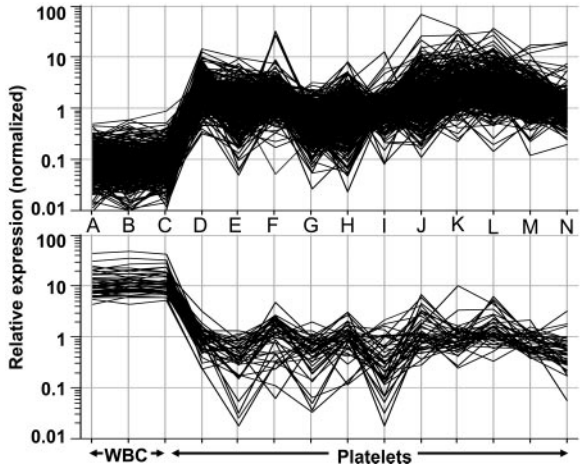

Figure 3. In silico gene rank–intensity plots from a first-generation platelet gene chip. Normalized data from individual microarray analyses obtained from 3 distinct leukocyte samples (A-C), 5 normal platelet samples (D-H), and 6 essential thrombocythemic platelet samples (I-N)[90] were analyzed by one-way ANOVA using parametric testing to identify a 432-member gene list. (top) Relative expression of the platelet-restricted genes (n = 389). (bottom) Expression intensity of the leukocyte-restricted genes (n = 43). Note the clear difference in the expression patterns between the 2 groups (leukocyte vs platelet).

Future directions and applicability

Custom platelet chips

The expense of global transcript and platelet protein profiling precludes investigators from performing larger disease cohort studies. The development of cost-effective and reliable tools for transcript profiling would facilitate platelet-related diagnostics and experimentation. Thus, we have fabricated a custom platelet microarray that can be used in conjunction with proteomic analyses to study normal and diseased platelets. The final transcript list was established by analyzing microarray results from normal and ET platelets[90] and included platelet-restricted genes without detectable expression in leukocytes, genes that appear to be associated with a thrombohemorrhagic ET phenotype, and genes whose platelet expression is more than 10-fold greater than its leukocyte expression or whose leukocyte expression is more than 10-fold greater than its platelet expression. Several *Arabidopsis* probe elements were included to serve as normalization controls and to minimize interslide and intraslide variability.[100] After removing duplicates, the final list contains 432 genes that clearly cosegregate by cell type (Figure 3). Although initial analyses demonstrate the potential of this chip to distinguish ET platelets from normal platelets (data not shown), further studies are ongoing. Nonetheless, the development of platelet-specific gene and protein chips could lead to more widespread applicability of this technology to platelet-related and thrombotic disorders.

General profiling perspectives

Future advances in proteomic technology that incorporate miniaturization,[101] coupled with an ability to integrate functional genomics and proteomics,[102] will help unravel the complex biological pathways that are relevant to platelet-associated disorders. The human genome encodes between 20 000 and 40 000 genes, whereas the estimated number of functional proteins may number as much as half a million because of alternative splicing, translational regulation, and posttranslational modifications.[102] Because of this large number of functional proteins, we anticipate that integrated analysis of the transcriptome and the proteome are required to optimally dissect the molecular mechanisms responsible for platelet-related diseases (Figure 4). Parallel advances in quantitative proteomic techniques will also have an impact on the field,[34-37] resulting in the identification of platelet-related disease biomarkers and novel therapeutic targets.

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Authorship

Contribution: D.V.G., P.L.P, and W.F.B. reviewed the literature and wrote the paper.

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Correspondence: Dmitri V. Gnatenko, Department of Medicine, Division of Hematology, T15-040 HSC, State University of New York, Stony Brook, NY 11794-8151; e-mail: dgnatenko@notes.cc.sunysb.edu.

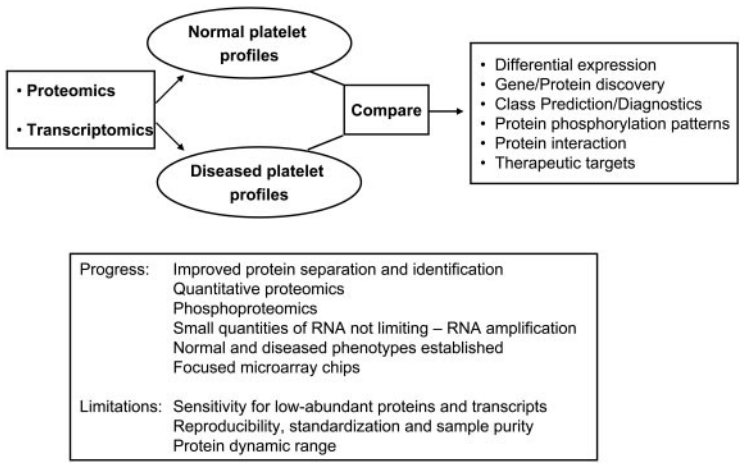

Figure 4. Integration of platelet transcript and protein profiling to study human diseases. The combination of proteomic and transcriptomic technologies can be applied for comparative studies between normal and diseased platelets, ultimately leading to novel diagnostic assays or to the identification of novel therapeutic targets. Potential applications include treatment of not only single-gene platelet disorders but also the broader subset of patients with platelet-related cardiovascular or cerebrovascular disease. The box summarizes current limitations and progress achieved to date.

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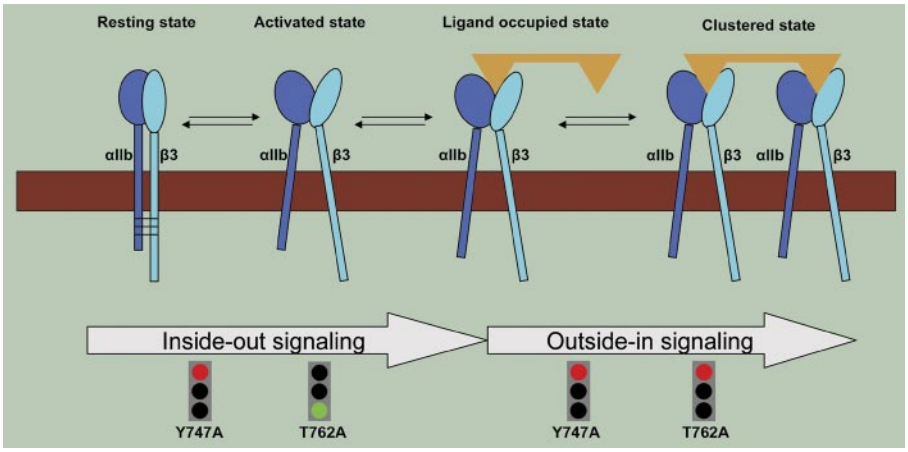

Distinction between inside-out and outside-in signaling across integrin αIIbβ3.

stabilizes the aggregate and supports responses, including platelet spreading and clot retraction. Hence, inside-out and outside-in signaling constitute the 2 elements of the bidirectional signaling across αIIbβ3 (see figure) and represent potential antithrombotic therapeutic targets, and their dissection is indispensable to our understanding of platelet biology.

The inside-out and outside-in signaling pathways ultimately trace to cytoplasmic tails (CTs) of αIIbβ3, which serve as receivers and transmitters of bidirectional signaling within the receptor. While the CTs of the αIIb and β3 subunits are short, they are structurally complex and interact with numerous binding partners. Ordinarily, the contribution of specific structural elements or individual amino acids can be dissected by straightforward mutagenesis. However, platelets are anucleated; and alternative approaches have had to be invoked to examine inside-out and outside-in signaling. These approaches have included the following: (1) expression of αIIbβ3 in heterologous cells; (2) transfection of the primary megakaryocytes; (3) introduction of membrane-permeable peptides into platelets; and (4) generation of knock-in mice expressing mutant αIIbβ3. Each of these approaches has been used successfully to probe integrin signaling, but each approach has inherent limitations.

By infecting fetal liver cells from β3-deficient mice with retrovirus engineered to encode wild-type β3-subunit and transplanting these cells into irradiated recipients, Zou and colleagues show that these cells can successfully express αIIbβ3 and rescue both inside-out and outside-in signaling in blood platelets. This strategy is then used to introduce specific mutations into the mouse platelets. Inside-out signaling is evaluated by the capacity of the platelets to bind soluble fibrinogen, and outside-in signaling is assessed by platelet spreading. A single point mutation in the midregion of the β3 CT, β3Y747A, prevents restoration of both inside-out and outside-in signaling, while a point mutation at the extreme C-terminus of β3, β3T762A, results in selective loss of outside-in signaling. These results are generally consistent with those derived from the other approaches we outlined and fortify the conclusion that different sites within the β3 CT can mediate different aspects of the bidirectional signaling.

The experimental approach for in vivo expression in platelets described by Zou et al here and by others[1] allows analyses of αIIbβ3 activation in platelets in vivo and has high throughput potential. Fetal liver transplantation could undoubtedly be applied to other platelet proteins provided that a deficient mouse background is available. Low, variable, and unstable expression of the platelet protein may set limitations on this approach. Nonetheless, the Zou et al paper sets the precedent for a new strategy to dissect inside-out and outside-in signaling across αIIbβ3 in vivo.

The authors declare no competing financial interests. ■

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HEMATOPOIESIS

Comment on Raslova et al, page 3225, and comment on Macaulay et al, page 3260

MEGAprofiles provide big insights into platelet function

Wadie F. Bahou STATE UNIVERSITY OF NEW YORK AT STONY BROOK

This issue of *Blood* contains reports from 2 groups that have used microarray profiling of CD34$^+$-differentiated hematopoietic stem cells to dissect molecular mechanisms regulating megakaryocytopoiesis and/or for identification of functionally novel platelet receptors. Both reports provide new insights into proplatelet formation and platelet function.

Hematologists are intrigued by megakaryocytes (MKs), hematopoietic cells readily distinguishable by their abundant cytoplasm and large size (~ 35-160 μm), nuclear polyploidy (up to 64N), and relative paucity (0.02%-0.05% of total nucleated bone marrow cells). While discovery of the existence and hemostatic functions of blood platelets is generally assigned to Bizzozero based on pioneering observations made during the 1880s, linking megakaryocytes to platelet biogenesis is credited to James Wright in 1906, who made conclusions based on histomorphometric similarities of granular and cytoplasmic content between both cell types.[1] In the subsequent century, considerable insight has been achieved in identifying receptors and signaling pathways that regulate platelet and megakaryocyte biology. Nonetheless, the genetic machinery controlling megakaryocyte transition into the distinctive endomitotic switch that precedes the epiphany of cytoplasmic maturation, membrane demarcation, proplatelet formation, and platelet release remains incompletely

understood. Similarly, the application of genomic technologies for identification of novel, functionally-important platelet genes and proteins remains a high priority.[2]

Raslova and colleagues describe cellular mRNA profiling to specifically dissect genetic changes occurring during an in vitro model of megakaryocyte differentiation and ploidization. Cells were flow-sorted by modal ploidy, and gene changes were compared between 2 cellular subsets: aggregate 2N+4N MKs versus aggregate 8N+16N MKs. Of interest, transcript changes were limited to approximately 350 genes across all the subsets, 106 of which were consistently down-regulated and 248 of which were consistently up-regulated between the 2 groups. Further analysis highlighted additional differences between the up-regulated and down-regulated subsets; specifically, members of the latter subset not infrequently (24/105) corresponded to genes involved in DNA replication (arrest) and recombination repair, while a majority of the former corresponded to genes important to platelet biogenesis, viability, and function (ie, actin and microtubule cytoskeleton, glycoproteins, and signaling/transport proteins). It is important to note, however, that when the gene subsets are carefully analyzed by gene ontology functional classification, considerable overlap exists between these 2 groups, confounding detailed interpretations. Nonetheless, the data do support a role of ploidization in modulating gene expression, although a direct, regulatory role in platelet biogenesis remains speculative.

The study by Macaulay and colleagues adapts a nearly-identical in vitro strategy of MK differentiation, coupled with a bioinformatic strategy to specifically identify novel transmembrane domain–containing MK receptor proteins. An initial gene list of 151 transcripts was assembled using paired, comparative expression profiling with $CD34^+$-differentiated erythroblasts, and the list further pared using strict criteria to identify putative, functionally-relevant platelet proteins. Five of 8 highly-selected genes were characterized by transcript and protein expression studies, 3 of which were shown to be platelet restricted *(G6b, G6f,* and *LRRC32),* and another of which *(SUCNR1)* encoded the G protein–coupled succinate receptor.[3] More detailed functional studies established that succinate (a key component of the citric acid cycle) exhibited costimulatory effects on platelet aggregation induced by various platelet agonists (adenosine diphosphate, thrombin receptor activating peptide, and a glycoprotein VI–specific collagen peptide). The latter functional data are especially insightful in that they identify a novel, cocoupling signal transduction pathway in platelets, while opening new avenues of research linked to platelet hyperreactivity.[4]

While both study designs overlap in their initial in vitro differentiation strategies using megakaryocytes, the conclusions, future directions, and ability to compare data sets are distinct and limited. One restriction inherent in cross-experimental microarray data-sharing is the disparate platforms used among investigators, well exemplified in these studies, that used nonoverlapping oligonucleotide or cDNA probe sets for their analyses.[5,6] This limitation does not minimize results, although it emphasizes the importance of validation strategies of transcript differences initially identified by microarray. The identification of a costimulatory succinate receptor on platelets represents a discrete end product of integrated MK transcriptomic studies, coupled with a concrete hypothesis and sophisticated experimental design to characterize novel functional receptors. Likewise, the application of microarray technology to dissect MK ploidization is highly novel, and although the results are less focused in scope, they are likely to yield broader implications in the foreseeable future. Finally, such unique data sets open up exciting opportunities for sophisticated data mining likely to provide unexpected insights into molecular mechanisms of MK and platelet function.[7]

The author declares no competing financial interests. ■

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IMMUNOBIOLOGY

Comment on León-Ponte et al, page 3139

Serotonin: a real blast for T cells

John Gordon and Nicholas M. Barnes UNIVERSITY OF BIRMINGHAM MEDICAL SCHOOL

Mouse T lymphocytes unexpectedly produce the classic neurotransmitter serotonin, which—upon binding the constitutively expressed 5-HT_7 G_s-coupled receptor-subtype—signals ERK1/2 phosphorylation and NFκB activation to boost their stimulation.

Serotonin/5-hydroxytryptamine (5-HT), often perceived as the brain's "happy chemical," is—increasingly—assuming a prominent role in immune regulation. In fact, this "neurotransmitter" is primarily a product of the periphery, with gut enterochromaffin cells the principal factories. Intestinal lymphocytes could certainly be exposed directly to their output, though platelets are typically proffered as the source of the monoamine within the immune system: these 5-HT–loaded reservoirs delivering their potent cargo at sights of inflammation and immunologic reactivity.[1,2]

In this issue of *Blood,* León-Ponte and colleagues not only consolidate serotonin's importance to T-cell activation but also tender a paradigm whereby the monoamine is provided by the immune cell itself. Critical to any outcome from 5-HT exposure—whether the source be autocrine or paracrine—is a capacity for target cells to sense the monoamine. This could be via the serotonin transporter (SERT) or one or more of 14 receptor subtypes (13 in

Platelet transcriptome and cardiovascular disease

Lisa Senzel†,
Dmitri V Gnatenko &
Wadie F Bahou

†Author for correspondence
State University of New York, Department of Pathology, Stony Brook, NY, USA
Tel.: +1 631 444 2601;
Fax: +1 631 444 2653;
lsenzel@notes.cc.sunysb.edu

Platelet hyper-reactivity is likely to play a role in cardiovascular disease, but there are no standardized tests to evaluate platelet responsiveness. A 'platelet chip' (a synthetic oligonucleotide microarray representing all platelet-restricted genes) is under development as a tool for high-throughput characterization of platelet-based bleeding and clotting disorders. In future, platelet gene profiling may be used to improve thrombohemorrhagic risk assessment and to guide antiplatelet therapy for patients at risk of cardiovascular disease.

Genetics & cardiovascular disease

Family studies have shown that there is a genetic component to coronary disease risk, and estimates of the effects of inheritance on myocardial infarction (MI) have ranged from 20 to 80% [1]. However, attempts to identify polymorphisms that influence coronary disease risk have yielded only weak associations thus far. Common polymorphisms for apolipoproteins and other genes involved in lipid metabolism are associated with odds ratios for MI ranging from 0.8 to 1.5 [2]. Even weaker associations with coronary disease are found for platelet glycoprotein (GP) receptor variants, factor V Leiden, prothrombin 20210A and factor VII polymorphisms, according to a recent meta-analysis [3]. Importantly, genetic predisposition for MI can result from an additive or synergistic effect of several genes that have only a small role by themselves [4]. Therefore, large studies focusing on gene–gene and gene–environment interactions are required for a better understanding of cardiovascular disease risk.

Role of platelets in atherothrombosis

Atherosclerosis is a chronic inflammatory disease specific to the arterial circulatory system [5], and platelet–leukocyte interactions play a role in this process. Platelets accumulating at sites of arterial injury can recruit leukocytes to the vessel wall via binding to platelet surface GPs. Activated platelets express CD40 ligand, and thereby provoke endothelial cells to secrete chemokines and express adhesion molecules. Dysfunctional endothelial cells promote platelet thrombosis by several mechanisms:

- Secreting von Willebrand factor, which causes platelet adhesion;
- Exposing and/or releasing platelet agonists, such as collagen, epinephrine, ADP and thrombin, thereby enhancing post-adhesive aggregation;
- Failing to produce platelet antagonists such as nitric oxide and prostacyclin (PGI_2).

The primary role of platelets is to trigger hemostasis in a damaged vessel to maintain vascular integrity. Platelets undergo adhesion and activation when a vessel wall is disrupted at the site of a wound, but will also undergo these changes when atherosclerosis disrupts a coronary artery. Thus, the function of normal platelets is usually too efficient for the safety of patients with coronary artery disease (CAD), and antiplatelet drugs have been designed to reduce platelet function [6]. In the causation of atherothrombosis, platelets are one player in a cast that includes neutrophils, monocytes, macrophages, endothelial cells and coagulation proteins. Just as antiplatelet drug therapy can be useful in conjunction with other approaches (i.e., cholesterol-lowering therapy), the development of platelet genetic profiling as a tool for risk assessment has great potential utility, although other risk factors are also important.

Antiplatelet drug therapy in CAD

Currently, the key antiplatelet drugs fall into three categories:

- Aspirin, which blocks the cyclooxygenase pathway;
- Thienopyridines such as clopidogrel, which inhibit the $P2Y_{12}$ receptor for ADP on platelets;
- GPIIb–IIIa antagonists such as tirofiban and eptifibatide.

Keywords: aspirin, cardiovascular risk, clopidogrel, coxibs, cyclooxygenase, GPIIb–IIIa antagonists, microarray, platelet, thienopyridines

future medicine part of fsg

For patients with preclinical CAD, who do not experience angina, antiplatelet therapy is recommended for men over the age of 50 or women over the age of 60 with at least one major risk factor for CAD: tobacco smoking, hypertension, diabetes mellitus, hypercholesterolemia or a family history of premature CAD. For these patients, aspirin at a dose of 75–162 mg/day is recommended, assuming there are no contraindications. Addition of low-intensity warfarin (target international normalised ratio of 1.3–1.5) may be considered for individuals who have multiple risk factors and extensive family history of premature CAD [6,7]. The Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) trial compared aspirin alone with a combination of aspirin and clopidogrel for patients with either documented cardiovascular disease or multiple atherothrombotic risk factors. The combination of aspirin and clopidogrel demonstrated a trend toward benefit, but there was no statistically significant difference in the combined incidence of MI, strokes and cardiovascular deaths, and there was a trend toward a higher incidence of severe bleeding in the dual therapy group [8]. Thus, for this group of patients, dual antiplatelet therapy did not appear superior to aspirin alone.

All three types of drug are useful in unstable CAD, including acute coronary syndromes and acute MI. For patients with stable coronary symptoms, oral aspirin is recommended unless there is a contraindication to aspirin, and then clopidogrel may be substituted. For secondary prevention in patients who have experienced an MI, the benefit of low-dose aspirin therapy exceeds its risks by an even wider margin than in primary prevention [7]. The role of clopidogrel in secondary prevention of CAD has received scrutiny in many recent studies and cost-analyses. An analysis based on the Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) study did not find clopidogrel to be cost-effective [9], but subsequent studies identified cost-effective indications for clopidogrel in patients who have experienced acute coronary syndromes [10,11].

Aspirin resistance & potential utility of platelet profiling

While clinical 'aspirin failure' refers to the recurrence of thrombotic events despite aspirin therapy, laboratory 'aspirin resistance' has no standardized definition. Residual platelet activation can be detected despite aspirin therapy, whether platelet activation is measured by aggregometry [12], flow cytometry for P-selectin [13], agglutination of fibrinogen-coated beads [14] or a platelet function analyzer [15]. Some studies suggest that laboratory aspirin resistance may predict recurrent thrombotic events [12,15]. However, aspirin resistance testing is not currently recommended in clinical practice, because there is insufficient evidence to guide the tailoring of antiplatelet therapy based on such results [16,17]. Differences in the pharmacokinetics of aspirin metabolism, use of other nonsteroidal anti-inflammatory drugs, presence of cyclooxygenase (COX)-1 polymorphisms, catecholamine levels, COX-2 expression and isoprostane synthesis may contribute to the differences in aspirin response. Standardized laboratory tests that can predict clinical aspirin failure are required. It is unclear whether aspirin-resistant patients would benefit from higher doses of aspirin, alternate antiplatelet agents or other approaches for cardioprotection. If platelet phenotypic/genotypic assays can be developed to predict responsiveness or adverse events, such assays would be clinically useful.

Cyclooxygenase inhibition & CAD

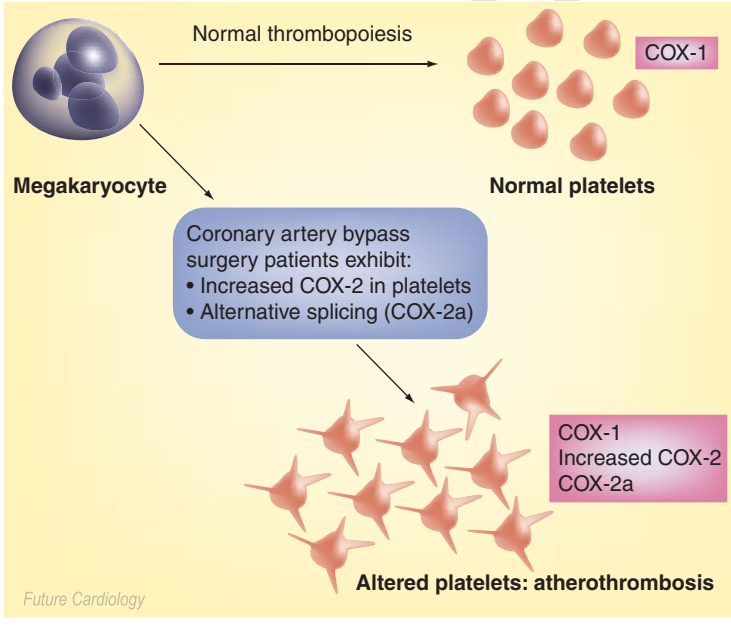
Aspirin inhibits COX activity by irreversibly acetylating the COX-1 enzyme. This inhibition concurrently diminishes the generation of both thromboxane $(Tx)A_2$ and PGI_2 from arachidonic acid. Platelets normally express only COX-1, not COX-2.

The cardiovascular risks of COX-2 inhibitors (coxibs) have recently drawn attention. Antman describes a model for the use of coxibs in the normal state and in states of vascular inflammation [18]. Central to this model is the balance between TxA_2 and PGI_2 in normal and diseased vessels. In the normal artery, the balance between PGI_2 and TxA_2 production favors PGI_2 and inhibition of platelet-dependent thrombus formation. In the atherosclerotic artery, the production of both PGI_2 and TxA_2 is increased, in part, owing to increased platelet activation with compensatory PGI_2 formation via both COX-1 and COX-2 in endothelial cells; the net effect is an imbalance favoring TxA_2 production and platelet-dependent thrombus formation. Low-dose aspirin selectively impairs COX-1-mediated TxA_2 production in platelets, restoring the net antithrombotic balance. In the setting of atherosclerosis, COX-2 has a greater role as a source of PGI_2 and more TxA_2 is produced; thus, inhibiting COX-2 has a more profound effect on prostanoid balance, favoring TxA_2 production and promoting platelet-dependent thrombosis.

The dogma that platelets express only COX-1, but not COX-2, has been challenged in certain scenarios, including CAD. COX-2 is expressed in young platelets [19] and in platelets from cardiac bypass patients [20]. An mRNA variant of COX-2, termed COX-2a, was found in platelets from patients after coronary artery bypass grafting [21]. Another variant, known as COX-3, may or may not be expressed in platelets [22,23]. One study reported *de novo* synthesis of COX-1 mRNA and protein by mature platelets *in vitro* [24]. The relative activity of the COX pathways, through which prostaglandins and thromboxanes are generated, clearly has an impact on the cardiovascular risk of the individual patient. However, methods with which to assess such activity are not currently in clinical use. Furthermore, more global approaches to dissect non-COX-mediated platelet pathways that may regulate thrombotic risk are generally lacking.

Figure 1 depicts the observation that platelets from patients undergoing coronary artery bypass grafting display increased levels of COX-2 mRNA and also express the splice variant COX-2a [21,23]. This example illustrates a model in which differences in platelet gene transcription contribute to atherothrombosis. As megakaryocytes differentiate into platelets, mRNA transcription results in different levels of gene expression and alternative splicing produces different sequences. These changes may be useful for identifying disease states and assessing thrombotic risk.

Figure 1. Model in which differences in platelet gene transcription contribute to atherothrombosis.



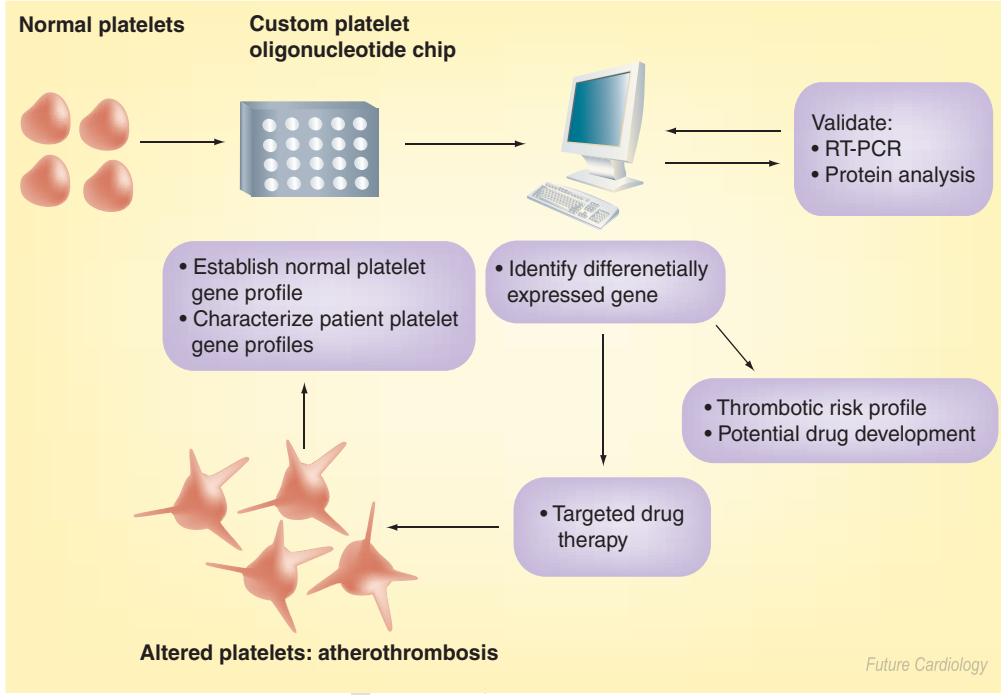
As megakaryocytes differentiate into platelets, mRNA transcription results in different levels of gene expression and alternative splicing produces different sequences. These changes may be useful for identifying disease states and assessing thrombotic risk. In this example, platelets from patients undergoing coronary artery bypass grafting display increased levels of COX-2 mRNA and also express a splice variant known as COX-2a [21,23].
COX: Cyclooxygenase.

Platelet transcriptome: the application of microarray analysis to platelets

Modern post-genomic, high-throughput approaches allow integrated studies of molecular components involved in cell function at the RNA and protein level. Platelets represent an attractive, simplified model for these studies as they lack nuclear DNA and their genome consists of a small subset of megakaryocyte-derived mRNA transcripts. This complete pool of platelet RNAs is significantly smaller than the transcriptome of a nucleated cell [25]. Platelets retain the capacity for protein biosynthesis from cytoplasmic mRNA. The traditional paradigm that platelet mRNA content is invariant and gradually declines with cell senescence was challenged when signal-dependent pre-mRNA splicing was identified in platelets [26]. Signal-dependent splicing provides a mechanism for altering the repertoire of translatable messages in response to cellular activation/stimulation. Furthermore, platelets have essential components of a functional spliceosome and selected unspliced pre-mRNAs. These spliceosomes retain a unique ability to splice pre-mRNA in the cytoplasm (as opposed to the typical nuclear location), a capability not described in any other mammalian cell. This discovery emphasizes that the molecular mechanisms of platelet function cannot be optimally dissected without accurate platelet transcript profiling [27].

Microarray analysis adapts artificially constructed grids of known DNA samples in such a way that each element of the grid probes for a specific RNA sequence; these are then used to capture and quantify RNA transcripts. Microarray platforms developed to date represent closed transcript profiling systems – that is, they detect only those transcripts that correspond to specific probes imprinted on the chip. Transcripts without corresponding probes are not detected. Recent technological advancements allow accurate whole genome transcript profiling and are capable of detecting alternatively spliced transcripts [28]. **Figure 2** outlines a three-step general

Figure 2. Schema outlining comprehensive approach for platelet microarray development.



The fabrication of a platelet-specific spotted oligonucleotide chip will provide a powerful tool for screening patient cohorts with cardiovascular disease. Once transcriptional differences are identified and validated, they will provide insight into the molecular basis of thrombosis, and can be used for the development of novel diagnostic therapeutic drug targets, improved thrombohemorrhagic risk profiling and a more tailored approach to antiplatelet drug prescribing.
RT-PCR: Reverse transcriptase PCR.

strategy for the development of platelet microarrays in clinical diagnostics, and incorporates the need for generation of a comprehensive platelet oligonucleotide array, determination of normal and reliable profiles and application of the arrays for diagnostics [29].

Serial analysis of gene expression (SAGE) represents an open transcript profiling system, which relies on the observation that short sequences (tags) within 3′-mRNAs can stringently discriminate among genes. Differentially expressed genes can be identified in a quantitative manner, and genes expressed at very low levels can be identified by SAGE. The overrepresentation of mitochondrial transcripts in the platelet transcriptome limits the applicability of SAGE in platelet diseases, but the technique is useful for gene discovery [27].

To date, a limited number of microarray experiments using platelet-derived mRNAs have been published; these studies generally agree on platelet transcript quantitation and gene expression patterns [25,30–32]. The feasibility of analyzing platelet transcripts from a single platelet donor with as little as 50 ng total platelet RNA has been demonstrated; this is readily obtained by peripheral venipuncture [30]. Recently, microarray analysis was used to identify genes that are differentially expressed in several platelet-related diseases, including essential thrombocythemia. These studies clearly establish the feasibility of platelet transcript profiling in identifying differentially expressed genes, characterizing novel platelet-expressed genes and elucidating the molecular signature of a disease with potential application for platelet diagnostics [27,33,34].

Recently, platelet transcriptomes were analyzed from patients with acute MI and stable CAD to identify candidate genes with differential expression. The strongest discriminators of ST-segment elevation MI (STEMI) were CD69 and myeloid-related protein (MRP)-14. Plasma levels of MRP-8/14 heterodimer were elevated in STEMI patients. A validation study found that MRP-8/14 levels could independently predict the risk of any vascular event in apparently

healthy women [33]. This study was criticized for nonagreement with microarray and proteomic studies by others, and for possible contamination of the platelet fraction by leukocyte microparticles [35]. The authors countered that they demonstrated MRP-14 expression in both megakaryocytes and platelets that lacked antibody staining for leukocyte markers. In any case, this study clearly used platelet expression profiling to identify a novel determinant of cardiovascular events, and this determinant was validated in one clinical study.

Integrating platelet transcriptomic & proteomic studies

Although studies have shown relatively good correlation between proteomic and transcriptomic data in the end points of detection and identification, there is sometimes a discordance between the two. The lack of detailed quantitative correlations limits the ability to compare platelet gene and protein levels with those of nucleated cells. Some studies have found that approximately one third of platelet proteins identified by proteomic methods are not reflected in the transcriptome [31,36–39]. The discordance may be due to the limited mRNA stability of these genes, failure of microarray analysis to detect very low levels of RNA, the occurrence of proteins that may be synthesized in megakaryocytes, after which mRNA is degraded, and the fact that some proteins may be taken up from plasma or from other cells rather than synthesized in megakaryocytes or platelets [39]. Conversely, mRNA transcripts identified by transcriptomic methods are not always detected by proteomic techniques; the frequency of this problem is unclear. When proteins are not identified, this may be due to the failure of current proteomic methods to identify proteins with certain structural or biochemical characteristics and/or to lack of translation of mRNA [39].

With improving technology, it is becoming feasible to adapt transcriptomic analysis to predict differences in the proteome, to validate these differences between normal and diseased platelets and to exploit these differences to develop modern diagnostic tests [27,40]. Using suppressive-subtractive hybridization PCR, one group found gender differences in platelet transcripts that were consistently reflected in protein levels [41].

There is no clear relationship between mRNA (or protein) levels and the importance of the resulting protein-to-platelet function. Dynamic changes in levels of rare transcripts can have more relevance to platelet function than absolute levels of common transcripts. For some genes and their corresponding proteins, substantial fluctuations in levels may be normal, whereas for others small changes may carry a large impact on platelet function. The level of redundancy of transcripts and the amplification of small signals by downstream transduction pathways are factors that contribute to the unequal relationship between abundance and functional significance.

Limitations & perspectives of transcript profiling

The major limitations of modern transcript profiling approaches include reliable and reproducible detection of low-abundant transcripts, feasibility of truly quantitative transcript profiling, bulky and complex data processing and, in the case of platelets, limited amounts of RNA. Furthermore, accurate platelet transcript profiling requires stringent attention to purification methodologies because a single nucleated cell (i.e., a leukocyte) contains considerably more mRNA than a platelet. The platelet transcriptome is complex and dynamically controlled at different levels, including regulation by miRNAs, signal-dependent pre-mRNA splicing and translational control pathways. Despite significant progress in microarray chip design, accurate transcript profiling still requires validation by quantitative (Q)PCR or other techniques, followed by protein antigenic and functional correlational analyses. Efficient mRNA amplification, development of more sensitive whole genome microarrays (which detect alternatively spliced transcripts) and enhancements to bioinformatics software should obviate some of these restrictions.

The expense of global transcript and platelet protein profiling precludes investigators from performing larger disease cohort studies. The development of cost-effective and reliable tools for transcript profiling would facilitate platelet-related diagnostics and experimentation. Thus, we have fabricated a first-generation custom platelet microarray that can be used in conjunction with proteomic analyses to study normal and diseased platelets. The final transcript list contains 432 genes that clearly cosegregate by cell type (platelet vs leukocyte) [27]. The development of platelet-specific gene (and protein) chips should lead to more widespread applicability of this technology to dissect mechanisms of platelet-related thrombotic disorders.

Conclusion & future perspective

Much is known about the mechanisms by which platelets contribute to atherothrombosis. However, this knowledge has yet to be translated to laboratory tests that can predict the risk of thrombotic events in CAD patients. Thus, no guidelines are available to tailor antiplatelet therapy to any measure of platelet responsiveness in patients.

The ability to integrate functional genomics and proteomics will help unravel the complex biological pathways that are relevant to platelet-associated disorders, resulting in the identification of platelet-related disease biomarkers and novel therapeutic targets. A custom platelet microarray is currently under development to assess thrombohemorrhagic risk in patients with platelet diseases. We would speculate that in the foreseeable future, platelet gene profiling will be developed as a tool to improve thrombohemorrhagic risk assessment and to guide antiplatelet therapy for patients at risk of cardiovascular disease.

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Executive summary

Genetics & cardiovascular disease

- Individually, polymorphisms for platelet glycoprotein receptor genes, apolipoproteins and other genes involved in coagulation and lipid metabolism show weak associations with coronary artery disease (CAD) risk.
- Genetic predisposition for myocardial infarction (MI) can result from an additive or synergistic effect of several genes that have only a small role by themselves.

Role of platelets in atherothrombosis

- Platelet–leukocyte interactions play an important role in the chronic inflammatory process known to be functional in atherosclerosis.
- The formation of a platelet plug at the site of an atheromatous plaque represents the final step in ischemic heart disease and MI.

Antiplatelet drug therapy in CAD

- Key antiplatelet drugs include aspirin, which blocks the cyclooxygenase pathway, thienopyridines such as clopidogrel, which inhibit the $P2Y_{12}$ receptor for ADP on platelets and glycoprotein IIb–IIIa antagonists such as tirofiban and eptifibatide.
- All are useful in unstable CAD, and aspirin or clopidogrel are used in primary or secondary prophylaxis in patients at cardiac risk.
- While aspirin alone is recommended in preclinical CAD, low-intensity warfarin may be added for individuals at very high risk of cardiovascular events. The role of clopidogrel in primary prevention requires further study.

'Aspirin resistance' & potential utility of platelet profiling

- Clinical 'aspirin failure' refers to the recurrence of thrombotic events despite aspirin therapy.
- Laboratory 'aspirin resistance' has no standardized definition.
- Standardized laboratory tests that can predict clinical aspirin failure are required.

Cyclooxygenase inhibition & CAD

- Aspirin inhibits cyclooxygenase (COX) activity by irreversibly acetylating the COX-1 enzyme, reducing the generation of both thromboxane (Tx)A_2 and prostacyclin.
- The atherosclerotic artery features an imbalance favoring TxA_2 production and platelet-dependent thrombus formation, an imbalance which is worsened by the use of COX-2 inhibitors.
- Platelets normally express only COX-1, but can also express COX-2 in certain clinical settings such as cardiac bypass.
- Methods to assess the activity of COX pathways in individual patients are not currently in clinical use.

Platelet transcriptome: the application of microarray analysis to platelets

- Although platelets are anucleate, they retain megakaryocyte-derived mRNA and are capable of translating protein.
- Platelets contain functional spliceosomes and carry out signal-dependent pre-mRNA splicing in the cytoplasm. Splicing may be a key regulatory event during platelet activation.
- Microarray analysis has demonstrated a clear and reproducible molecular signature unique to platelets.
- Transcript analysis is a tool for identifying novel platelet genes that may regulate normal and pathologic platelet functions.
- A 'platelet chip' (a synthetic oligonucleotide microarray representing a subset of platelet-expressed genes and their polymorphisms) would be useful for high-throughput characterization of platelet-based disorders.

Conclusion

- In future, platelet gene profiling may be used to improve thrombohemorrhagic risk assessment and to guide antiplatelet therapy for patients at risk of ischemic cardiovascular disease.

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Affiliations

- *Lisa Senzel, MD, PhD*
State University of New York, Department of Pathology, Stony Brook, NY, USA
Tel.: +1 631 444 2601;
Fax: +1 631 444 2653;
lsenzel@notes.cc.sunysb.edu
- *Dmitri V Gnatenko, PhD*
State University of New York, Department of Medicine, Stony Brook, NY, USA
Tel.: +1 631 444 1260;
Fax: +1 631 444 7530;
dgnatenko@notes.cc.sunysb.edu
- *Wadie F Bahou, MD*
State University of New York, Department of Medicine, Stony Brook, NY, USA
Tel.: +1 631 444 2059;
Fax: +1 631 444 7530;
wbahou@notes.cc.sunysb.edu